REVIEW PAPER

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Vitamin E as promising adjunct treatment option in the combat of infectious diseases caused by bacterial including multi-drug resistant pathogens – Results from a comprehensive literature survey

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ABSTRACT

The use of antibiotics has provoked an emergence of various multidrug-resistant (MDR) bacteria. Infectious diseases that cannot be treated sufficiently with conventional antibiotic intervention strategies anymore constitue serious threats to human health. Therefore, current research focus has shifted to alternative, antibiotic-independent therapeutic approaches. In this context, vitamin E constitutes a promising candidate molecule due to its multi-faceted modes of action. Therefore, we used the PubMed database to perform a comprehensive literature survey reviewing studies addressing the antimicrobial properties of vitamin E against bacterial pathogens including MDR bacteria. The included studies published between 2010 and 2020 revealed that given its potent synergistic antimicrobial effects in combination with distinct antibiotic compounds, vitamin E constitutes a promising adjunct antibiotic treatment option directed against infectious diseases caused by MDR bacteria such as *Pseudomonas aeruginosa*, *Burkholderia cenocepacia* and methicillin-resistant *Staphylococcus aureus* (MRSA). In conclusion, the therapeutic value of vitamin E for the treatment of bacterial infections should therefore be investigated in future clinical studies.

INTRODUCTION

Antibiotic resistance as a global threat in the combat of infectious diseases

Since the implementation of antibiotics has been established as one of the major pillars in medicine, many avenues have been opened in the combat of bacterial infections. In the course of the commercialized use of antibiotic compounds global health is now being challenged with another upcoming threat given that the emergence of multi-drug resistant (MDR) bacterial pathogens can be found not only in the hospital environment but also in community settings [1, 2]. The rise and dissemination of these MDR bacteria appear as evolutionary responses to the application of antibiotics in order to escape from the antimicrobial attacks [1, 2]. The genetic plasticity that is fundamental for the development of resistance mechanisms can be achieved by either mutational adaptation or acquisition of foreign DNA through horizontal gene transfer, for instance [1, 3]. Changes in the bacterial response to antibiotic challenge resulting from distinct genetic alterations can lead to resistance against the attacking drug. The effectiveness of antibiotics can be reduced by (i) molecular modifications of the antimicrobial target subsequently decreasing its affinity, (ii) by compromising the drug uptake, (iii) by alterations in influx and efflux mechanisms in order to reduce the concentration of the drug within the bacterial microorganism, and (iv) by

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induced changes in metabolic pathways [1, 4–8]. Due to the dynamic development and genetic variability in bacterial resistance mechanisms that can be acquired, it is of utmost importance to prevent and control the spread of antibiotic resistance on a global level [8].

According to the "Global Priority List of Antibiotic-Resistant Bacteria to Guide Research, Discovery, and Development of New Antibiotics", published by the World Health Organization (WHO) in 2017, most critical MDR bacterial species such as *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacteriaceae* are considered the most critical ones and therefore rated of first priority whereas *Staphylococcus aureus* and *Salmonella* species (spp.), for instance, are set to priority level two and *Streptococcus pneumoniae* to priority level three [9]. Infectious diseases caused by MDR bacterial pathogens are not only emerging worldwide but are also associated with higher mortality rates as compared to diseases caused by bacterial strains that are susceptible to antibiotics [10, 11]. While the WHO published a "Global Action Plan on Antibiotic Resistance" in 2015 in order to tackle antibiotic resistance [12] and global research has more intensely focused on strategies to combat the afore-mentioned as well as further MDR bacterial species, there is still a lack of sufficient antimicrobial treatment options [13]. Therefore, the pharmacological research focus has shifted to alternative antibiotic-independent approaches in order to avoid the ongoing trigger for acquisition of resistance in bacterial strains due to inappropriate antibiotic application and to maintain treatment of bacterial infections in the future [7, 14].

Vitamin E as a promising candidate molecule in the combat of infectious diseases

Basic molecular features of vitamin E. Vitamin E constitutes a group of lipophilic molecules which can be classified into tocopherols, tocotrienols and tocomonenols all of which consisting of a polar head group and a hydrophobic isoprenoid side chain [15, 16]. In humans, the main biologically active form of vitamin E is α-tocopherol [17]. Vitamin E is an essential, fat-soluble nutrient whose major dietary sources are vegetable oils and plant seeds including commonly used nuts [15, 16]. The intestinal absorption of vitamin E is governed by the same mechanisms as for dietary fats: it is secreted in chylomicron particles and triacylglycerol, phospholipids and cholesterol and transported via the lymphatic system to peripheral tissues where it is taken up into the cell upon lipoprotein-receptor binding [15, 18].

Immune-modulatory properties of vitamin E. The earliest and most detailed investigated features of vitamin E were its antioxidant properties, since the molecule serves as peroxyl, oxygen, and superoxide anion radical scavenger protecting polyunsaturated fatty acids in membranes and lipoproteins [2, 17, 19]. Besides its antioxidant properties, recent studies revealed that vitamin E can modulate the host immune responses and impact inflammatory processes in experimental *in vivo* models and humans in both health and disease [15–17, 20]. Vitamin E deficiency has been shown to impair baseline cellular and humoral immune responses [21–26], whereas both animal and human studies provided evidence that vitamin E supplementation above current dietary recommendations even enhanced the host immune functions [27, 28]. In particular, vitamin E is capable of modulating T cell proliferation and interleukin-2 production. It enhances lymphocyte proliferation responses, natural killer cell activity, heterophil- and monocyte-oxidative burst and furthermore, reduces prostaglandin E2 production [16, 17, 29]. In addition, vitamin E was shown to regulate the activity of distinct enzymes including protein kinase C which is involved in cell-mediated immune responses and cell proliferation [17, 30] These health beneficial, immune-modulatory properties exerted by vitamin E are currently investigated in more detail, particularly in cardiovascular and neurodegenerative morbidities, cancer and infectious diseases [17, 31–33].

Evidence for antimicrobial effects of vitamin E. According to previous *in vitro* and *in vivo* investigations, distinct vitamins such as vitamin D and vitamin C exhibited antimicrobial properties [34, 35]. Vitamin D, for instance, displayed antibacterial effects against *Porphyromonas gingivalis* [36], *Streptococcus mutans* [37], and *Helicobacter pylori* [38]. In addition, synergistic antimicrobial effects of vitamin D in combination with distinct antibiotic compounds directed against MDR strains of *P. aeruginosa* and *S. aureus* have also been described [39]. Furthermore, in an acute campylobacteriosis model, vitamin D application could sufficiently dampen pro-inflammatory immune responses upon peroral infection of mice with the enteropathogen *Campylobacter jejuni* [34]. Besides vitamin D also vitamin C has been proven as potent antimicrobial agent against *C. jejuni* [40–43], *H. pylori* [44, 45] and *Mycobacterium tuberculosis* [46, 47]. These findings support the hypothesis that also other vitamins including vitamin E might be promising candidate molecules with antimicrobial modes of action. In support, a study from 1995 revealed that a derivate of tocotrienol extracted from the leaves of *Tovomitopsis psychotriifolia* could inhibit growth of both Gram-positive and Gram-negative bacterial species such as *S. aureus*, *Bacillus cereus* and *P. aeruginosa*, respectively [48]. Previous reports further documented beneficial effects of vitamin E in combating infectious diseases such as respiratory infections and chlamydiosis [29, 49–51] as well as bacterial infections caused by *Escherichia coli* [52, 53] and *H. pylori* [54]. Moreover, the water-soluble derivate of vitamin E, α-tocopheryl-polyethylene-glycol-succinate (TPGS), has been established for the application of various nanocarriers in the context of drug delivery [55, 56]. Because of its amphiphilic structure, TPGS is an ideal molecule in various drug delivery systems, including prodrugs, micelles, liposomes and nanoparticles. Furthermore, TPGS has been used as an absorption enhancer, emulsifier, solubilizer, permeation enhancer and stabilizer [57–59]. Recent studies investigated not only the enhancement of drug delivery by TPGS itself, but also its direct antimicrobial effects due to its ability to

cause perturbations in the integrity of bacterial cell membranes, facilitating the penetration of antimicrobial agents [60]. Given these heterogeneous molecular and multifaceted antimicrobial effects of vitamin E, we here performed a comprehensive literature survey focusing on recently investigated antibacterial and immune-modulatory effects of vitamin E and its derivates, particularly against MDR bacterial pathogens.

MATERIAL AND METHODS

Inclusion and exclusion criteria

Inclusion criteria were *in vitro* and *in vivo* studies that addressed the evaluation of antibacterial and immune-modulatory effects of modified and non-modified vitamin E as well as investigations on possible synergies of vitamin E with antibiotics and other agents in order to assess adjuvant effects of tocopherol for antibacterial treatment regimens. Due to the limited research that has been conducted so far, clinical trials have been excluded as well as *in vitro* studies investigating the antimicrobial effects of vitamin E directed against viruses and fungi.

Search strategy

A structured literature search was performed including articles that have been published in PubMed between 2010 and June 2020 and that investigated the effects of vitamin E directed against bacteria including MDR pathogens. By using the Boolean logic through the advanced search history on the PubMed database, the following steps were undertaken in order to find relevant publications.

At first, the database was searched for publications with the key word "vitamin E". To ensure that also publications with synonyms and variations of the term "vitamin E" were considered, we included the key words "tocopherol", "tocotrienol" and "tocomonoenol" into our search by using the Boolean operator "OR". Secondly, the term "antimicrobial OR antibiotic OR immune-modulatory" was used to ensure that the studies identified were focusing on the antimicrobial and immune-modulatory properties of vitamin E. Thirdly, we added the term "antibiotic resistance" to the query to assure that studies on MDR bacteria were included as well. Finally, to limit the spectrum of results, all three terms were united through the Boolean operator "AND" in the following manner: "(vitamin E OR tocopherol OR tocotrienol OR tocomonoenol) AND (antimicrobial OR antibiotic OR immune-modulatory) AND (antibiotic resistance)".

Thereby, 21 studies could be found of which 15 qualified for full-text analyses after reading the abstract. By evaluating all 15 publications regarding the inclusion criteria, some articles were withdrawn given that they did not focus on the antimicrobial effect of vitamin E, another one because it was related to viruses. Two further articles had to be excluded since full text access was not possible. Finally, seven studies fulfilled the inclusion criteria and were reviewed in detail here.

Data extraction

In order to assess the studies systematically, they were reviewed with regard to the inclusion and exclusion criteria as well as to the scientific method, targeted bacteria (Gram-negative and Gram-positive), controls and main findings.

RESULTS

Findings from included studies

An antibiotic bioassay revealed that the Bacterial Lipocalin Protein (BcnA), which is released by the Gram-negative and MDR bacterial species *Burkholderia cenocepacia* increases antibiotic resistance by sequestering bactericidal antibiotics into the extracellular space. Experimental and X-ray crystallographic structure-guided computational analyses and ligand docking modeling examined the binding modes of BcnA [61]. While the tested antibiotics such as norfloxacin, rifampicin, ceftazidime, and gentamicin interacted with the rim of the lipocalin, more lipophilic structures such as α-tocopherol bound to the interior of the lipocalin tunnel. In Nile-Red displacement assays α-tocopherol exhibited an insufficient mean binding inhibition constant, whereas the mean binding inhibition constant of the tested antibiotics were two to four orders of magnitude higher, indicating that α-tocopherol had a superior binding affinity as compared to the applied antibiotics. To test the BcnA-inhibitory activity of α-tocopherol *in vivo*, *Galleria mellonella* larvae were infected with the *P. aeruginosa* PAO1 strain after treatment with BcnA in order to simulate the MDR mechanism caused by the BcnA-mediated antibiotic binding. Following co-incubation of ten larvae without or with 10 µM α-tocopherol the survival of *G. mellonella* larvae was examined 20 h post-infection. In the α-tocopherol treated group a significantly higher survival rate as compared to the untreated control group could be assessed that was comparable to the survival rate of another control group that was not treated with resistance-causing BcnA but only challenged with *B. cenocepacia*, suggesting that α-tocopherol counteracts the resistance caused by the antibiotic binding by BcnA [61].

Another study assessed the minimal inhibitory concentration (MIC) of the third generation cephalosporin ceftazidime and the fluorquinolone norfloxacin alone or in combination with TPGS against the BcnA-producing *B. cenocepacia* K56-2 strain applying the broth microdilution assay [62]. In combination with TPGS, the MICs of norfloxacin and ceftazimide were eight- and four-fold reduced, respectively, as compared to MICs of the antibiotic compound alone. Similar results were observed when challenging *P. aeruginosa* PAO1 strain, resulting in an over three-fold reduction for both norfloxacin and ceftazidime. Furthermore, the effect of TPGS in the BcnA-producing *B. cenocepacia* K56-2 strain exposed to sub-inhibitory antibiotic concentrations, defined as 25% of the MIC, were examined and surviving bacteria were counted after a two-hour and six-hour challenge with norfloxacin or polymyxin B. After 2 h, the combination of norfloxacin and TPGS

resulted in an approximately 50% reduction in the number of surviving *B. cenocepacia* K56-2 bacterial cells as compared to norfloxacin alone. The 24-h TPGS-norfloxacin co-challenge also resulted in a significant growth rate reduction of the bacterial strain compared to treatment with norfloxacin only. Results after challenge with polymyxin B alone and in combination with TPGS for 2 h did not differ, whereas the combinatory challenge for 6 h caused a significant reduction in the bacterial survival rate compared to the one determined following exclusive polymyxin B application. In this study, the *in vivo G. mellonella* infection model was used as well in order to assess the BcnA-inhibitory activity of TPGS. The survival rate of the larvae that had been infected with the BcnA-producing *B. cenocepacia* K56-2 bacteria in the presence of TPGS was significantly higher than in the absence of TPGS. In a further experiment, larvae were infected with BcnA-producing *B. cenocepacia* K56-2 strains and treated with either norfloxacin, TPGS, or their combination. When compared to the norfloxacin- or TPGS-only treatment regimens, the survival of BcnA-producing *B. cenocepacia* K56-2-infected larvae increased to more than 80% upon treatment with norfloxacin in combination with TPGS. A similar experiment was conducted with *P. aeruginosa* PAO1-infected larvae also resulting in a significant increase of the larvae survival rate when treated with norfloxacin and TPGS together compared to exclusive norfloxacin application [62].

In a study from 2016, synergistic effects of antibiotics in combination with vitamin E were evaluated against the Gram-negative *A. baumannii* and Gram-positive methicillin-resistant *S. aureus* (MRSA) by the Kirby-Bauer disk diffusion assay [63]. A control study of antibiotic susceptibility revealed that *A. baumannii* was resistant to the eight tested antibiotic compounds (namely, imipenem, piperacillin/tazobactam, polymyxin B, doripenem, meropenem, amikacin, ofloxacin and aztreonam), while a combination of the antibiotics with vitamin E showed synergistic effects. An increase of the zone of inhibition could be found when vitamin E was combined with imipenem as well as with piperacillin/tazobactam, resulting in a significant reduction of the respective MIC. In consequence, *A. baumannii* was found to be susceptible to both antibiotics in synergy with vitamin E. Even though the inhibition zones of the six other antibiotics (i.e., polymyxin B, doripenem, meropenem, amikacin, ofloxacin and aztreonam) were moderately enlarged upon co-application of vitamin E, these synergistic effects did not result in antibiotic susceptibility of *Acinetobacter baumanii*.

Furthermore, MRSA was included into the assay and found to be resistant to applied antibiotics such as moxifloxacin, oxacillin, rifampicin and oxytetracycline and susceptible towards linezolid only. Co-application of vitamin E with moxifloxacin, oxacillin, rifampicin and oxytetracycline, however, did not improve the inhibitory effect of the antibiotics, and MRSA remained resistant. But when vitamin E was used in combination with linezolid, the susceptibility of MRSA to linezolid could be further improved as indicated by an enlarged zone of inhibition [63].

The modifying effect of vitamin E on the antibiotic activity of the aminoglycosides amikacin, neomycin and gentamicin was investigated in an *in vitro* study, determining the MICs of the aminoglycosides in sub-inhibitory concentrations (MIC/8, MIC/4, MIC/2) when combined with α-tocopherol against clinical MDR *P. aeruginosa*, *E. coli* and *S. aureus* isolates applying the broth microdilution method [60]. Treatment with vitamin E alone did neither exhibit any antimicrobial effects against the bacterial strains, nor did vitamin E have any impact on the antibiotic effects of the aminoglycosides when given at concentrations according to their MIC. The addition of vitamin E to sub-inhibitory concentrations of the antibiotics, however, resulted in an increase in the synergistic effects between α-tocopherol and the aminoglycosides. At MIC/2, a significant reduction of the MIC of respective aminoglycosides, namely neomycin, amikacin and gentamicin, was determined in all tested bacterial strains. At MIC/4, the synergistic actions of vitamin E and both amikacin and neomycin significantly reduced the MIC against *P. aeruginosa*. Furthermore, the MIC against *E. coli* was lowered by the combination of vitamin E and neomycin at MIC/4. Remarkably, α-tocopherol decreased the antibiotic activity of neomycin or gentamicin against *S. aureus* and *E. coli* which also held true for *P. aeruginosa* when the vitamin was combined with neomycin, indicative for antagonistic effects [60].

Another study investigated the effect of α-tocopherol on the severity of *P. aeruginosa*-induced pneumonia [64]. Firstly, confluent rat microvascular endothelial cells were pre-treated with α-tocopherol, then exposed to the wild-type strain of *P. aeruginosa* PAK, testing whether α-tocopherol could prevent *P. aeruginosa*-induced increases in lung endothelial permeability. α-Tocopherol treatment alone did not impact the paracellular permeability, whereas *P. aeruginosa* challenge increased it five-fold. Pre-treatment with α-tocopherol significantly decreased *P. aeruginosa* mediated increases in endothelial paracellular permeability. This increase in permeability and also the induction of actin stress fiber formation are primarily mediated by GTPase-protein Ras homologue A (RhoA) and plasminogen activator inhibitor 1 (PAI-1) which are activated by *P. aeruginosa* [64]. RhoA and PAI-1 levels were measured in the supernatants of rat microvascular endothelial cells and rat alveolar epithelial cells that had been pre-treated with α-tocopherol and exposed to *P. aeruginosa* for 6 h. Pre-treatment with α-tocopherol could significantly reduce the activation of both RhoA and PAI-1 and their effects. Another experiment using Western blots showed that α-tocopherol was also able to block the insertion of specific bacterial exo-enzymes into alveolar epithelial cells and therefore prevented the mediation of several bacterial activities such as increasing vascular permeability and impairing microtubule and microfilament stability. Furthermore, in a murine *P. aeruginosa*-induced pneumonia model, mice were treated with α-tocopherol via intraperitoneal injections before and after *P. aeruginosa* instillation. The α-tocopherol application decreased lung permeability, reduced colony forming units of *P. aeruginosa* and myeloperoxidase release, resulting in a significantly reduced mortality in *P. aeruginosa* infected mice [64].

The effects of vitamin E in combination with arginine on the immune response and clearance of *Salmonella* was investigated in another study in broiler chickens [20]. Therefore, one-day-old chicks were assigned to four groups, each of them receiving a different diet starting immediately after birth: antibiotic-free diet (negative control), antibiotic-supplemented diet (positive control), antibiotic-free diet plus arginine and vitamin E (AVE), or antibiotic-free diet plus arginine, vitamin E and mannan-oligosaccharide (AVM). On day 3 or day 7, the chicks were infected with a *Salmonella* Typhimurium strain. Heterophil- and monocyte-oxidative burst and lymphocyte proliferation, antibody titers, and cecal *Salmonella* burdens were measured at defined time points post-infection. When infected on day 7, the AVE- and AVM-dietary effects on the activity of monocyte-oxidative burst were rather heterogeneous, indicating that due to the already established microbiota the *Salmonella* infection was not sufficient to generate a prominent immune response. When challenged as early as day 3, however, the results turned out to be more consistent. Seven days after challenge birds fed the AVE diet displayed a significantly higher heterophil-oxidative burst as compared to the birds fed the positive control or the AVM diet, and more pronounced lymphocyte proliferation compared to the other groups. Moreover, on 14 days post-infection, birds from the AVE-diet cohort exhibited more pronounced heterophil- and monocyte-oxidative bursts as well as lymphocyte proliferation versus the birds from the other treatment regimens. The addition of mannan-oligosaccharide did not further enhance these effects but rather restricted the immune-modulatory actions of arginine and vitamin E. Of note, in these experiments the AVE diet neither affected *Salmonella* antibody titer nor the pathogen burdens in the ceca after challenge [20].

In another study β-Ga$_2$O$_3$:Cr^{3+} nanoparticles modified with intercellular adhesion molecule-1 (ICAM1) -antibody-conjugated TPGS (I-TPGS/Ga$_2$O$_3$) were prepared as a novel antibiotic carrier for tigecycline for the treatment of pulmonary infection caused by tigecycline-resistant *K. pneumoniae* (TRKP). The TRKP strain was challenged with either Ga$_2$O$_3$/tigecycline, free tigecycline or I-TPGS/Ga$_2$O$_3$/tigecycline and subsequently, both the MIC and the minimal bactericidal concentrations were evaluated [55]. Both parameters were highly reduced when using the I-TPGS/Ga$_2$O$_3$/tigecycline nanoparticles as compared to the other treatments. The antibacterial activities were further examined *in vivo* applying an acute pneumonia mouse model utilizing either tigecycline-sensitive *K. pneumonia* (KPN) or TRKP. After oral challenge with either KPN or TRKP, the mice were subjected to the respective formulations. In both groups I-TPGS/Ga$_2$O$_3$ was the only treatment resulting in a 100% survival rate as opposed to the other treatment regimens. Moreover, the bacterial colony forming numbers assessed in the murine lungs of the I-TPGS/Ga$_2$O$_3$/tigecycline group were significantly lower as compared to the other groups. In order to investigate the role of TPGS in more detail, the expression levels of efflux pumps-related genes of TRKP after treatment with TPGS/Ga$_2$O$_3$, ICAM1-TPGS or TPGS alone were assessed applying the real-time

reversed transcriptase-polymerase chain reaction (RT-PCR) method, showing a significant decrease of expression in all three groups compared to the untreated control group and thus indicating an inhibitory effect of TPGS on the multi-drug-efflux pump activity of the bacteria [55].

Further findings from studies unraveling the multifaceted properties of vitamin E

Vitamin E as enhancer of targeted drug delivery. TPGS has already been shown to improve the drug delivery of vitamin E itself and is established as a widely used drug delivery carrier and excipient carrier for enhancing the retention times and stability of the drug [55,56,65,66]. The potential of TPGS as promising synergistic antimicrobial agent against MDR bacteria was further supported by an *in vitro* study preparing a nanocarrier for the antibiotic compound capuramycin SQ641 that was applied to *M. tuberculosis* infected murine macrophage-like cells [67]. Different tetra-hydrofuran solutions were used in the preparation of the capuramycin SQ641 nanocarriers. A nanocarrier formulation with added vitamin E produced a more effective capuramycin SQ641 reservoir and resulted in a more pronounced drug accumulation in the cell. Furthermore, a three-compound combination of capuramycin SQ641, cyclosporine A (serving as a drug efflux inhibitor) and vitamin E inhibited intracellular replication of *M. tuberculosis* more effectively than the capuramycin SQ641-cyclosporine A combination, resulting in a three-fold enhanced reduction of viable *M. tuberculosis*. Vitamin E was further shown to decrease the polarity of the nanocarrier core, resulting in a spatial overlap of the two drugs intracellularly and subsequently, in enhanced drug efficacy [67].

Inhibitory effects of vitamin E on efflux pumps in bacterial and cancer cells. Several studies assessed the inhibitory effects of vitamin E on bacterial efflux-pumps which displays another direct antimicrobial property that can be utilized to overcome MDR through targeted drug delivery. In one study the authors reported that α-tocopherol is able to modify fluoroquinolone resistance in *C. jejuni* and *Campylobacter coli* isolates by modifying the bacterial efflux-pump activity resulting in reduced MICs of different fluoroquinolone agents [65]. The inhibiting effect of vitamin E on efflux-pump activity was not only addressed in bacteria, but also in cancer cells. In one study the targeted delivery strategies of the cytostatic drug doxorubicin was investigated [57]. Cancer cells are known to acquire resistance to doxorubicin through high levels of efflux-pump activity, subsequently reducing intracellular retention of doxorubicin resulting in diminished activity of the compound. To assess whether TPGS could reduce the doxorubicin resistance by altering drug efflux, the doxorubicin efflux activity was tested in human ovarian carcinoma cell lines towards rhodamine-123 as efflux-pump substrate in either absence or presence of TPGS. Treatment with TPGS resulted in a significant increase in rhodamine retention due to a reduced efflux compared to untreated ovarian carcinoma cells, suggesting that integrating TPGS into a mixed-micelle
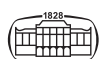
drug-delivery system could enhance the cytotoxic effects of doxorubicin [57].

Vitamin E and biofilms. Results from another *in vitro* experiment testing strategies to overcome antibiotic resistance mediated by biofilm production revealed that modified vitamin E in form of anionic α-tocopherol phosphate liposomes self-assembled into structures showed antibacterial effects and had the ability to inhibit the growth of *Streptococcus oralis*. Furthermore, α-tocopherol phosphate co-transmitted with the cationic electrolyte Tris augmented the penetration into oral multi-species biofilms and supported the bactericidal effect of α-tocopherol phosphate [68].

DISCUSSION

Main findings of the literature survey

The comprehensive literature survey presented here revealed antibacterial properties of vitamin E against distinct Gram-negative species as shown both *in vitro* and *in vivo*. Particularly the actions of vitamin E for overcoming lipocalin BcnA-induced resistance against *B. cenocepacia* and other lipocalin-producing bacterial species such as *P. aeruginosa* were described [61, 62]. Other direct antibacterial effects against *P. aeruginosa* [64] and, moreover, antibacterial actions against *K. pneumonia* [55] could be found. While vitamin E supplementation alone did not exhibit clinically relevant antibacterial activities, antimicrobial susceptibility of MRSA towards linezolid could be enhanced when vitamin E was added. The combination of vitamin E with imipenem as well as with piperacillin/tazobactam was sufficient to even overcome antibiotic resistance of *A. baumannii*, resulting in susceptibility for both antibiotic compounds. Further potent synergistic effects were found for vitamin E in combination with aminoglycosides in sub-inhibitory concentrations against *P. aeruginosa*, *E. coli* and, less distinctly, against *S. aureus* [63]. This *in vitro* study also revealed rather contrasting results, given that a combination of vitamin E and gentamicin as well as neomycin even induced a decrease in antimicrobial activity [63]. Furthermore, the high potential of vitamin E to enhance targeted drug delivery was shown to result not only from enhanced antibiotic effects due to its molecular structure but also from its direct antimicrobial properties [55]. Hence, vitamin E might be considered as a promising antibacterial agent particularly in form of an adjuvant for several antibiotic compounds and also as a potential immune-modulatory agent improving the host immune responses upon bacterial challenges [20].

Open questions and future research

In this literature survey the antimicrobial and immune-modulatory properties of vitamin E were evaluated in technically very heterogeneous model systems in studies of limited numbers *in vitro* and *in vivo*. Therefore, definitive conclusions regarding the clinical use of vitamin E in combating MDR bacteria should be drawn with caution. Nevertheless,

considering the complexity of potential health-beneficial effects exerted by vitamin E, it is of utmost importance to further investigate their underlying molecular mechanisms in more detail. Based on the diverse antimicrobial and adjuvant properties of vitamin E that were discovered in the past ten years, it is necessary to perform well-designed studies to determine the antimicrobial and immune-modulatory properties of vitamin E in the future. The effects of vitamin E could be distinguished to "direct antimicrobial actions" and "properties that enhance antibacterial activity of a distinct antibiotic compound". Although only a small amount of experiments investigated the direct antimicrobial effect of vitamin E alone, promising results were found against *P. aeruginosa* induced pneumonia, for instance [64]. Notably, all of the direct vitamin E-induced antibacterial effects could only be found when challenging Gram-negative bacteria. Given these findings, research for effective vitamin E dependent antibacterial therapies should rather focus on Gram-negative than Gram-positive pathogens. In any case, the ability of vitamin E to counteract antibiotic resistance by binding bacterial lipocalin proteins and, moreover, the inhibitory effect on efflux pump activity poses vitamin E as possible excipient for overcoming MDR, since it was shown that vitamin E does not only reduce the resistance level of MDR tumor cells [57, 69, 70], but also of distinct bacteria such as *K. pneumoniae* [55], *C. jejuni*, and *C. coli* [67] by inhibiting their efflux pump activity.

In most afore-discussed experiments, vitamin E was combined with synthetic antibiotics or other antimicrobial compounds and gained promising results by increasing antibacterial activity not only against non-MDR Gram-negative and Gram–positive bacterial strains, but also against MDR strains [60, 62, 63]. The potent synergistic effects of vitamin E in combination with antibiotics that were demonstrated by valid results from *in vitro* studies point towards a high potential of the molecule in combating antibiotic resistance and should be conducted in more detail applying both *in vitro* and *in vivo* studies. Not only synergistic effects of vitamin E with conventionally used antibiotics but also with other compounds were discovered in the past ten years. An experimental study from 2013, for instance, developed a synthetic polycationic polymer incorporating vitamin E and therefore significantly enhanced antimicrobial activity against *S. aureus*, *E. coli*, and in co-delivery with antibiotics also against MDR *P. aeruginosa* [71]. Endogenously produced antimicrobial peptides and polycationic polymers as their synthetic mimics pose as effective agents against microbes [72–75]. These macro-molecules exhibited multiple mechanisms to produce a microbial killing effect and are able to act upon the entire cellular membrane through electrostatic interactions and subsequent pore generation [76], while antibiotics only act on specific molecular targets within the bacterial organisms and therefore ease their acquisition of resistance [1, 77]. Moreover, antimicrobial peptides are able to disrupt the bacterial membrane and thus, alter its permeability which can be utilized for a co-delivery with other antimicrobial agents such as antibiotics [73, 76]. The authors of the afore-mentioned study hypothesized that incorporation

of vitamin E in cationic polycarbonates may enhance anti-microbial activity as a result of the amphiphilicity of the polymers [71]. To further confirm vitamin E as a potent compound for polycationic polymers these findings should be addressed in more depth.

In conclusion, given the lack of knowledge regarding the exact molecular mechanisms and the limited number of bacterial species on which the investigations of the antibacterial properties of vitamin E were performed and particularly considering antagonistic effects that were obtained as well [60], more detailed experimental studies should be performed in order to find effective strategies for combating bacterial infections and eventually overcoming multi-drug resistance in a vitamin E (co-)dependent manner. Regarding the dynamics in the emergence and dissemination of MDR bacterial strains [10, 11], it is also of high importance to find ways to successfully transfer the *in vivo* results that have already been obtained into clinical research trials.

Even though this survey revealed only one study assessing immune-modulatory effects of vitamin E that met all inclusion criteria, the capability of vitamin E to modulate the immune responses upon bacterial challenge is another promising area for future research. Other experimental including clinical studies as mentioned in the introduction revealed immune-modulatory (e.g., anti-inflammatory) properties of vitamin E that may provide highly appreciated additional effects in the combat of bacterial infections by dampening the induced pro-inflammatory immune responses. However, both *in vitro* and *in vivo* studies are needed to better understand the molecular mechanisms underlying the immune-modulatory modes of actions exerted by vitamin E that may be advantageous in the combat of infectious diseases caused by bacterial and particularly by MDR pathogens.

Limitations of the literature survey

Given the relatively small numbers of the reviewed papers and the variety of experimental approaches and set-ups, conclusions about the antimicrobial and immune-modulatory properties of vitamin E should be rather carefully drawn. The search strategy was performed as precise as possible and the studies were analyzed and evaluated carefully. However, the studies' quality assessment was carried out by a single investigator, thus, research mistakes cannot be precluded and there might be relevant studies that were not considered.

Ethics statement: Not applicable (literature survey).

Conflict of interests: SB and MMH are Editorial Board members.

ABBREVIATIONS

AVE	antibiotic-free diet plus arginine and vitamin E
AVM	antibiotic-free diet plus arginine, vitamin E, and mannan-oligosaccharide
BcnA	bacterial lipocalin protein
ICAM-1	Intercellular adhesion molecule 1
I-TPGS/Ga$_2$O$_3$	β-Ga$_2$O$_3$:Cr^{3+} nanoparticles modified with ICAM1-antibody-conjugated
KPN	tigecycline-sensitive *Klebsiella pneumoniae*
MDR	multidrug-resistant
MIC	minimal inhibitory concentration
MRSA	methicillin-resistant *Staphylococcus aureus*
PAI-1	plasminogen activator inhibitor
RhoA	Ras homologue A
RT-PCR	real-time reverse transcriptase-polymerase chain reaction
TPGS	α-tocopheryl-polyethylene-glycol-succinate
TRKP	tigecycline-resistant *Klebsiella pneumoniae*
WHO	World Health Organization

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